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CLINICAL OUTCOME OF INTRAVENTRICULAR IMPLANTATION AUTOLOGOUS ADIPOSE DERIVED NEURAL PROGENITOR CELLS IN PARKINSON

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Abstract–Parkinson's disease (PD) involves the malfunction and death of vital nerve cells in the brain, is a chronic and progressive movement disorder. Supportive medications and surgery may conduct, but no optimal results have been obtained. The main goal of this study was to investigate the effectiveness of the intraventricular implantation of adipose derived neural progenitor stem cells in post-Parkinson's disease patients. 12 patients were included in this study. Small adipose tissue was isolated by small lipopectomy under local anesthesia, cultured and derived become neural progenitor cells. Intraventricular implantation was performed in the operating room. The evaluation was carried out using the Unified Parkinson's Disease Rating Scale (UPDRS), include non-motor experiences and motor experiences of daily living, motor examination, and motor complications. The primary target was the UPDRS over the time period of 12 months after treatment as the end point. Descriptive statistics are provided. 10 of 12 patients (83.33%) had a significant improvement in mentation, behavior and mood, activity of daily living, and motor examination after treatment. There were no serious adverse events reported, limited to mild headaches, fever or vomiting, and all side effects resolved within few days. Because of the small sample size and non-randomised trial performed, we could not reach a definitive conclusion regarding the potential of intraventricular implantation. However, this study shows that repeated intraventricular implantation of autologous stem cells is advantageous.

INTRODUCTION

Parkinson's disease (PD) involves the malfunction and death of vital nerve cells in the brain, is a chronic and progressive movement disorder. The most obvious are shaking, rigidity, slowness of movement, difficulty with walking, thinking problems, depression, and anxiety. In 2015, PD affected 6.2 million people and resulted in about 117,400 deaths globally (NINDS, 2016; GBD, 2016). Parkinson's disease typically occurs in people over the age of 60, of which about 1% are affected. Males are more often affected (Carrol *et al.,* 2016; Kalia *et al.,* 2015). The average life expectancy following diagnosis is between 7 and 14 years (Sveinbjornsdottir, 2016). The cause of PD is generally unknown, but believed to involve both genetic and environmental factors. Non-motor symptoms, which include autonomic dysfunction, neuropsychiatric problems, sensory, and sleep difficulties, are also common (Kalia *et al.,* 2015; Jankovic, 2008). The motor symptoms of the disease result from the death of cells in the substantia nigra, a region of the midbrain. This results in not enough dopamine in these areas (NINDS, 2016). The reason for this cell death is poorly understood, but involves

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the build-up of proteins into Lewy bodies in the neurons (Kalia *et al.,* 2015).

Several neuroprotective agents have been developed to prevent brain tissue damage after Parkinson's disease. Initial treatment for PD is typically with the anti-parkinson medication levodopa (L-DOPA) with dopamine agonists (Samii *et al.,* 2004). As the disease progresses, these medications become less effective while at the same time they produce a complication marked by involuntary writhing movement (Sveinbjornsdottir, 2016). When oral medications are not enough to control symptoms, surgery, deep brain stimulation, subcutaneous waking day apomorphine infusion and enteral dopa pumps may be of use. This stage presents many challenging problems requiring a variety of treatments for psychiatric symptoms, orthostatic hypotension, bladder dysfunction, and more (Olanow *et al.,* 2011). Surgery to place microelectrodes for deep brain stimulation has been used to reduce motor symptoms, but it is more invasive and full of risks.

In the last 10 years, alternative approaches to restoring neural function after Parkinson's disease have been developed using the concept of neurorestoration using stem cell therapy (Bhasin *et al.,* 2011). Stem cells are multipotent progenitor cells that have been shown to have regenerative as well as imunomodulatory and growth stimulating properties. They have been shown in vitro to have the capacity to induce angiogenesis and differentiate into different cells types including cells of the nervous system. Stem cell treatment for Parkinson's disease is designed to target these neurons and help with the creation of new dopamine producing neurons. In addition, stem cells may release natural chemicals called cytokines which can induce differentiation of the stem cells into dopamine producing neurons (Najm *et al.,* 2011; Lee YH *et al.,* 2011).

Stem cell research has the potential to significantly impact the development of disease-modifying treatments for Parkinson's disease, and considerable progress has been made in creating dopamine-producing cells from stem cells. Cell models of Parkinson's disease generated from stem cells could help researchers screen drugs more efficiently than in currently available animal models, and study the underlying biological mechanisms associated with Parkinson's disease in cells taken from people living with the disease. Young et al reported that all subjects with Parkinson's disease were honed in on the salient variables include cognition, depression, sleep, and adjustment, and showed an improvement using stem cell (Young HE *et al.,* 2013).

The main goal of this study was to investigate the effectiveness of the intraventricular implantation of adipose derived neural progenitor stem cells in post- Parkinson's Disease patients and evaluate using the Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) before and after stem cell implantation. The MDS-UPDRS has four parts, namely, I: Non-motor Experiences of Daily Living; II: Motor Experiences of Daily Living; III: Motor Examination; IV: Motor Complications (Goetz *et al.,* 2007). Adipose tissue-derived stem cells are considered to be ideal for application in regenerative medicine, e.g. Parkinson's disease. They can be easily and repeatable harvested using minimally invasive techniques with low morbidity. Adipose tissue-derived stem cells are multipotent and can differentiate into various cell types of the tri-germ lineages, including osteocytes, adipocytes, neural cells, vascular endothelial cells, cardiomyocytes, pancreatic â-cells, and hepatocytes. Interestingly, adipose tissue-derived stem cells are characterized by immunosuppressive properties and low immunogenicity. Their secretion of trophic factors enforces the therapeutic and regenerative outcome in a wide range of applications (Laura *et al.,* 2016).

MATERIALS AND METHODS

Subjects

This study was following the regulatory guidelines of the country. The patients were included if they had confirmed by two neurologists. Prior to the study, informed consent documents, details of the medical treatment and other necessary approval documents were delivered to all patients after full explanation of the procedure and the safety issues involved.

Twelve patients were included in this study. The evaluation was carried out using the Unified Parkinson's Disease Rating Scale (UPDRS). The scales include (1) non-motor experiences of daily living (13 items), (2) motor experiences of daily living (13 items), (3) motor examination (18 items), and (4) motor complications (6 items). The primary target was the UPDRS over the time period of 12 months after treatment as the end point. Descriptive

statistics are provided. The following inclusion and exclusion criteria were used for the patient (Table 1).

Procedure and Implantation Techniques

Isolation and intraventricular implantation of adipose derived neural progenitor stem cells were performed in the operating room. Autologous adipose tissue isolation was performed under local anaesthesia, and aspiration was performed with a sterile procedure.

Neural progenitor cells was derived from autologous adipose tissue. Small adipose tissue was isolated by small lipopectomy under local anesthesia after isolation then cultured and derived become neural progenitor cells for around 3 weeks. Before used, neural progenitor cells was validated. Characterization of neural progenitor cells by expression of L-Dopa with immunocytochemistry and expression of Notch using flow cytometry (NPCP technique by Purwati).

Under general anaesthesia, patients were conditioned in a supine position. The hair was shaved just behind the right frontal hairline, and the area was washed with antiseptic solution. A mark was made on the right Kocher point. A 2.5-cm wide linear incision was made in layers through the periosteum. The process was continued by creating a burr hole in the calvaria and a small dural incision. An Ommaya reservoir was inserted into the ventricle, then a maximum of 5 cc cerebrospinal fluid was slowly aspirated through the Ommaya reservoir with a wing needle. Stem cells were transplanted with the same wing needle (2×10^6 cells in 3cc normal saline) and flushed with 2cc normal saline. The surgical wound was then sutured layer by layer.

For booster implantation, the same procedures were performed without the open procedure or general anaesthesia one month after the first implantation. Hair did not need to be shaved, disinfection with povidone-iodine was performed at the skin and stem cell injection was carried out with the same dose using wing needle no. 25 through the subcutaneous transplanted Ommaya reservoir. Booster implantation was done twice at one-month intervals.

RESULTS

There were 12 subjects in this study, and all subjects were male. The youngest patient was 53 years old, and the oldest was 77 years old. Ten of the 12 patients had a significant improvement after stem cell therapy (83.33%) according to their improvement in mentation, behavior and mood, activity of daily living, and motor examination. Further details on patient characteristics and improvements are shown in Table 2.

DISCUSSION

Stem cells, including adipose tissue-derived stem cells, have emerged as a key element of regenerative medicine therapies due to their ability to differentiate into a variety of different cell lineages. Their capacity of paracrine secretion of a broad selection of cytokines, chemokines, and growth factors make them highly clinically attractive. Adipose tissue-derived stem cells have been shown to have the capacity as anti-apoptotic, anti-inflammatory, immunomodulatory, anti-scarring effects, and proangiogenic, which make these cells promising candidates for cellular therapy in regenerative medicine (Laura *et al.,* 2016; Bertolini *et al.,* 2012).

Brain is control center of the body. This organ has a wide range of responsibilities from coordinating our movement to manage on emotion, the brain does it all. For almost hundred years, it has been a mantra of biology that brain cell do not regenerate so need to add new neuron when the brain injured. In this study, the source of neural progenitor cells

Table 1. Inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
- Parkinson's disease patients severe - Aged 40 to 80 years - Parkinson's Subjects will not currently be experiencing dementia (DSM-IV criteria) - MMSE 20 or greater - No active infection/disease	- Subjects with severe hepatic impairment, COPD, galactorrhea and/or prolactin sensitive tumors - Parkinsonism due to Parkinson's-plus diagnoses or to medication - Subjects with a communicable disease, include HIV, Hepatitis - Subjects having deep brain stimulation

Table 2. Data progress of post-treatment patients

No.	Gender (M/F)	Age (years old)	48-Weeks Evaluation Pre-UPDRS*	Post-UPDRS*	Significant Improvement
1.	M	65	4	2	• Activity of daily living improved → speech, handwriting, eating, cutting food • Hallucinations and delusions decreased • Motor examination improved → facial expression, rigidity, finger taps
2.	M	68	4	3	• Activity of daily living improved → sleep problems, cognitive impairment, speech • Motor examination improved à finger taps, hand movements
3.	M	53	3	2	• Activity of daily living improved → daytime sleepiness, eating, handwritting • Motor examination improved → facial expression, arising from chair, finger taps, hand movements
4.	M	77	4	4	-
5.	M	73	4	2	• Activity of daily living improved → speech, handwriting, eating, cutting food, dressing • Hallucinations and delusions decreased Motor examination improved → facial expression, rigidity, finger taps, hand movements, balance walking
6.	M	68	3	2	• Activity of daily living improved → sleep problems, cognitive impairment, speech • Motor examination improved → facial expression, finger taps, hand movements
7.	M	70	4	3	• Activity of daily living improved → daytime sleepiness, eating, handwritting • Motor examination improved → facial expression, finger taps, hand movements
8.	M	66	3	2	• Activity of daily living improved → sleep problems, cognitive impairment, pain and other sensations, speech • Motor examination improved → facial expression, finger taps, hand movements
9.	M	74	3	2	• Activity of daily living improved → sleep problems, depressed mood • Motor examination improved→facial expression, finger taps, hand movements
10.	M	66	3	2	• Activity of daily living improved → eating, cognitive impairment, speech, hanwritting • Motor examination improved → facial expression, finger taps
11.	M	68	4	4	-
12.	M	66	3	2	• Activity of daily living improved → daytime sleepiness, eating, handwritting • Motor examination improved → facial expression, arising from chair, finger taps, hand movements

*MDS-UPDRS Score: 0 = Normal, 1 = Slight, 2 = Mild, 3 = Moderate, 4 = Severe

we used from autologous adipose tissue by small lipopectomy, because neural progenitor cells high expressed from adipose derived compared with from bone marrow derived, with expression of Notch and L-Dopa (Brito *et al.,* 2012; Purwati *et al.,* 2017).

There is no standardised dose for stem cell therapy associated with the route of administration and the type of disease. For example, an overly high dose in intraparenchymal implantation can affect the nutrition of grafted cells and, if given intravascularly, cause micro-emboli and vessel occlusion (Wang *et al.,* 2004). In this study, we used the dose of 2 x 10^7 stem cells with the intraventricular route applied directly into the intracranial space. This route makes the dose adjustment is more flexible, because it can be controlled by reducing the ventricular fluid if necessary based on the transplant dose. The risk of increased intracranial pressure and mass effects of the body can also be avoided. This dose was administered in 3 ml of fluid to avoid highly concentrated doses and excess fluid volume. No complications, such as signs of increased intracranial pressure, infections or seizures were observed.

The ventricular system has thin walls composed of ependymal cells. The permeable properties of ependymal cells make it quite effective for the treatment of certain medicines, including stem cell therapy targeting the brain parenchyma (Bordey *et al.,* 2006; Kazania *et al.,* 2009). On the lateral ventricle, the ventricular walls are surrounded by the subventricular zone (SVZ), which continuously produces new neurons (Rosenbaum *et al.,* 2007). The location of the neurogenic niche area is very close to the lateral ventricle, which explains why the administration of stem cells through the intraventricular route is an effective method for stem cell therapy in this study. The lateral ventricles are easy to access, enabling direct stimulation of the SVZ. Moreover, cerebrospinal fluid is the endogenous regulatory factor of neuronal differentiation in neural regeneration, where the plexus choroideus produces substances during brain development or the regeneration process after brain injury (Falcao *et al.,* 2012).

The results in all subjects showed no decrease in neurological status and no complications associated with the actions and effects from stem cells. Some possible side effects that could be observed after treatment are increased intracranial pressure, seizures, infection and rejection reaction by the body. However, this study demonstrated that this technique is safe and reported no complications. One other advantage, the presence of the reservoir, facilitates repeated injections when applying booster therapy.

There are several effective mechanisms of action involved, including neural cells regeneration, neurons direct stimulation, and trophic paracrine mediators. There is evidence that growth factors like stem cell may help improve brain regeneration (Palisano *et al.,* 2006). Adipose tissue may generate neurons and other supportive cells. Transplanted adipose-derived neural progenitor cells infiltrate the brain and may help regenerate new elements or combat the neurodegenerative process, fibrosis, and oxidative insults. Neuroprotection may involve release of several neurotrophic factors, that work through paracrine and/or-autocrine interactions.

The Unified Parkinson's Disease Rating Scale (UPDRS) is a comprehensive questions assessment of both motor and non-motor symptoms associated with Parkinson's Disease (Goetz *et al.,* 2007). The advantages of the UPDRS include its wide utilization, its application across the clinical spectrum of Parkinson's disease, its nearly comprehensive coverage of motor symptoms, and its clinimetric properties including reliability and validity. There is currently no cure for Parkinson's disease; several treatments have focused on relieving the symptoms. Current treatments include the use of oral preparations of L-3,4-dihydroxyphenylalanine (L-DOPA) and dopamine receptor agonists, apomorphine in more serious cases, continuous intestinal infusion of L-DOPA, and deep brain stimulation (DBS) in subthalamic nucleus and globus pallidus by using surgically implanted electrodes (Parisa *et al.,* 2015).

The underlying pathogenesis of Parkinson's disease is not fully understood, that's why developing new disease modifying therapies remains difficult. The ultimate idea is to "neuroprotect" and, in so doing, to interfere with the underlying pathogenic mechanism of nigral cell death and/or rescue damaged but still viable cell neurons. The motor and non-motor symptoms of this disease presumably would be arrested and possibly reversed if stem cells were utilized (Young *et al.,* 2013). In this study, autologous adipose-derived neural progenitor cells have the potential to revolutionize the treatment of disease by targeting dysfunctional tissues and to repair damaged tissues

without the use of immunosuppressive therapy, thereby making new treatments possible without significant adverse side effects.

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Potential Conflict of Interest

The authors declare that there is no conflict of interest.

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